Initial Characterization of Osteoblast Differentiation and Loss of RUNX2 Stability in the Newly Established SK11 Human Embryonic Stem Cell-Derived Cell Line.

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Public Summary:
We describe a novel model for investigation of genetically normal human osteoblasts in culture. Availability of culture models for molecular investigation of genetically normal human osteoblasts is important because differences between murine and human osteoblasts, demonstrated here for the regulation of Matrix Gla Protein

Scientific Abstract:
We describe a novel model for investigation of genetically normal human osteoblasts in culture. SK11 is a clonal progenitor cell line derived from human embryonic stem cells. Initially selected based on the expression of chondrogenic markers when differentiated in micromass culture, SK11 cultures display typical mRNA expression patterns of bone phenotypic genes under osteogenic conditions. These include Osterix, alpha1(I) collagen, Alkaline phosphatase, Osteonectin, Osteopontin and Osteocalcin. Similar to well-characterized murine osteoblast cultures, the osteoblast master regulator RUNX2 was present during the first few days after plating, but the protein disappeared during the first week of culture. Loss of RUNX2 expression is considered an important regulatory feature for osteoblast maturation. Indeed, following approximately 2 weeks of differentiation, SK11 cultures exhibited robust calcium deposition, evidenced by alizarin red staining. We also introduced a lentiviral vector encoding doxycycline (dox)-inducible FLAG-tagged RUNX2 into SK11 cells. Dox-mediated enhancement of RUNX2 expression resulted in accelerated mineralization, which was further increased by co-treatment with BMP-2. Like the endogenous RUNX2, expression of the virally coded FLAG-RUNX2 was lost during the first week of culture despite persistent dox treatment. By following RUNX2 decay after dox withdrawal from day-5 versus day-3 cultures, we demonstrated a developmentally regulated decrease in RUNX2 stability. Availability of culture models for molecular investigation of genetically normal human osteoblasts is important because differences between murine and human osteoblasts, demonstrated here for the regulation of Matrix Gla Protein, may have significant biomedical implications. J. Cell. Physiol. (c) 2014 Wiley Periodicals, Inc.